EXERCISE 7: AUTOMATED HEMOGLOBIN TESTING

Skills: 30 points
Objectives:

1. Define the following: hemoglobin, anemia, polycythemia, sulfhemoglobin, carboxyhemoglobin
2. State two possible causes of low and high hemoglobin values.
3. State the principle of the HemoPoint H2 hemoglobin procedure.
4. State six criteria which must be considered when setting up the HemoPoint photometer.
5. List 3 sample types and 3 anticoagulants which may be used for testing.
6. State the storage requirements and time limits for use of venous and arterial samples.
7. Describe the testing which must be performed for monitoring quality assurance in hemoglobin testing.
8. State when a "blank reading" of a microcuvette is required.
9. State the frequency of testing the "control cuvette" and the importance of only using the control cuvette designated for each instrument.
10. State the proper handling and storage of cuvettes including: temperature, amount of time they can be used after opening and the information to be placed on the container the first time opened.
11. List 8 limitations of the hemoglobin test procedure.
12. State what must be done if hemoglobin values are equal or greater than 23.5 g/dL.
13. State the normal values for hemoglobin levels for adult females, adult males, children and infants.
14. Perform and record the blank reading and testing the control cuvette for the HemoPoint H_2.
15. Perform and record the control solutions results for the test.
16. Evaluate the control cuvette and control solutions using predetermined criteria to determine if the instrument can be used for patient testing.
17. Perform an effective capillary puncture.
18. Perform a quantitative measurement of hemoglobin on whole blood using the HemoPoint H2 instrument.
19. Evaluate the patient results and determine whether they fall within the normal range.
20. State the time limit for testing the sample in the microcuvette.
21. Record the results using proper units.
22. After use, perform maintenance on the housing unit, touch screen, cuvette holder and optical unit.
23. State the length of time before the equipment can be used after cleaning the optical unit.
24. State the time limit for using the optical cleaner after removal from the foil pouch.

Discussion

Hemoglobin is the oxygen–carrying pigment and main component of red blood cells. Low hemoglobin levels may indicate anemia, recent hemorrhage or fluid retention. Elevated hemoglobin levels may indicate hemoconcentration from polycythemia or dehydration.

The **Hemo**Point® **H2** system is intended for the quantitative determination of hemoglobin (Hgb) in whole blood of adults, infants, and children in a professional point-of-care setting. It consists of a dedicated photometer and individual, single-use microcuvettes filled with reagents. Using a single drop of blood, Stanbio's HemoPoint® H2 offers accurate results for both hemoglobin and hematocrit (calculated) tests—in less than a minute. The easy-to-read touch screen displays the current time, battery status and allows for easy operation.

Using the microcuvette, a small amount of arterial, venous or capillary blood is taken up by capillary action. The filled cuvette is inserted into the **Hemo**Point® **H2** photometer, the color produced by the

chemical reaction in the cuvette is measured, and the Hgb value is displayed. The microcuvettes are intended to be used only once and must be disposed after use as potentially infectious waste, in accordance with the current regulations applicable to your establishment.

The **Hemo**Point® **H2** system is designed for use in medical practices and in clinical laboratories to assist in medical diagnostic investigations. In addition it can be used in emergency and intensive care units and in medical facilities such as blood banks.

This test system has been approved for use in laboratories with Waived status as defined in 452 CFR 493.15(c)(a).

Principle of the Test Procedure

In the **Hemo**Point® **H2** system, the use of microcuvettes with short light pathways makes it possible to analyze undiluted blood. The filled microcuvette is inserted into the **Hemo**Point® **H2** photometer, the color produced by the chemical reaction in the cuvette is measured, and the hemoglobin level is calculated and displayed. For this purpose, light is directed through the blood sample and the absorption is measured. From the amount of light absorbed by the sample, the concentration of the hemoglobin in the cuvette can be calculated.

Setting up the Photometer

Select a suitable place for setting up the photometer, according to the following criteria:

1. Avoid direct sunlight
2. Avoid strong electromagnetic fields
3. Avoid direct influence from ionizing radiation
4. Avoid rapid temperature fluctuations (keep away from heaters, open windows, ventilators, fans or air conditioning, etc.)
5. Avoid wet areas (i.e. wash basins)
6. Allow the photometer to reach room temperature! *Changing from a cold to a warm environment, (i.e. after storage or transport) condensation can form both on the inside and the outside of the photometer. Wait at least 1 hour, before you connect the photometer to a power supply.*

Place the photometer on a level counter adjacent to a power socket. Make sure there is enough room for the cuvette holder to be freely accessible.

Sample Collection and Preparation

The **Hemo**Point® **H2** Photometer can be used with capillary, venous, or arterial blood. Use EDTA, heparin or heparin/fluoride as anticoagulants, preferably in solid form, to avoid dilutional effects. Venous and arterial blood samples may be used if the blood collected is not more than 24 hours old and the samples have been stored refrigerated 35 – 46ºF (2-8ºC).

Prepare stored samples for measurement as follows:

1. Remove sample tube from the refrigerator and bring it to room temperature.
2. Mix the sample tube well. (i.e. by a mechanical rotator or hand inversion at least 10 times).

Quality Control

The **Hemo**Point® **H2** AutoCheck performs an internal check of the photometer's optic system every time the cuvette holder is opened. The following checks are performed each day of use:

1. The control cuvette supplied with the photometer can be used for a simple check of the photometer's calibration.
2. Use of external controls to assure that the microcuvettes and the photometer are performing correctly.

Blank reading

All the optical and mechanical components involved with the measuring cycle are carefully manufactured with extremely small tolerances. This is essential for the proper function of the photometer.

Always carry out a blank reading whenever you have removed the cuvette holder (i.e., cleaning, replacing with a new cuvette holder). This serves to reset the instruments electronics.

Control Cuvette

The control cuvette supplied with the photometer can be used for a simple check of the photometer's calibration. The testing quality of the photometer should be checked once daily using this control cuvette.

The Hemoglobin value and the permitted deviation of the control cuvette are stated on the storage box label. All photometers have a specific calibration due to the tolerances in the mechanical and electronic components. The control cuvette is only calibrated for the instrument with which it is delivered, i.e. the Hgb value stated on the storage box label is only valid for that one photometer, and could lead to completely different results on another photometer.

If you have several **Hemo**Point® **H2** *photometers, keep track of each control cuvette for each photometer. When not being used, keep the control cuvette in the original storage box. It is optimally protected there against breakage and contamination.*

External Quality Control

Controls, when assayed as actual specimens, help a laboratory evaluate whether a given procedure is performing with acceptable accuracy and precision. Laboratory Practices recommend the daily use of external controls to assure that the microcuvettes and the photometer are performing correctly. For this purpose, we recommend the use of Stanbio **Hemo**Point® **H2** Hemoglobin Controls.

Three (3) different control preparations are provided in the set. These are prepared from unfixed, stabilized human erythrocytes containing normal, low and high levels of hemoglobin.

With the help of these three (3) different concentrations you can easily assess the precision of the system.

There are a variety of hemoglobin controls on the market today. See package insert for suitability of use with the HemoPoint H2 meter.

Thoroughly mix the controls before use (see the control package insert for directions). *Compare the values obtained with the controls against the stated reference values. If the Hgb result falls outside the range of the controls, there is evidently a problem. Please consult Section 7 "Troubleshooting" in this regard*

Storage of Cuvettes

The microcuvettes consist of a clear body with a cavity which takes up approximately 10uL of blood which combines with the dry reagent chemistry. The optical distance between the cuvette walls is fixed and permits photometric determination of hemoglobin in undiluted blood samples.

1. Store the cuvettes in the original container at room temperature 15-30C (59-86F). DO NOT refrigerate.
2. Cuvettes are stable for **3 months** after opening the vial when stored at room temperature.
3. Write the date the vial was opened and your initials on the container label in the space provided.
4. Only remove ONE microcuvette at a time from the container and then immediately close the lid.
5. It is crucial not to touch the optical eye of the cuvette with fingers, dirty or sharp objects.

Limitations of the Procedure

1. The microcuvette sample can be measured immediately or within 10 minutes at the latest. After 10 minutes false results may be obtained.
2. Air bubbles in the optical eye, caused by inadequate filling of the microcuvette cavity, may cause false results. Discard the microcuvette and take another sample using a new microcuvette.
3. Ensure that you do not hold the microcuvette at its filling end, this may contaminate the optical eye preventing an accurate reading.
4. To avoid contamination of the cuvette holder, remove surplus blood from the outside of the microcuvette.
5. Always place the cuvette right side up in the holder. Placing the cuvette upside down can lead to erroneous results.
6. All results above 23.5 g/dL or equivalent must be confirmed by laboratory methods.
7. Sulfhemoglobin (a form of hemoglobin found in the blood in trace amounts that contains an irreversibly bound sulfur molecule that prevents normal oxygen binding.) Cannot be measured by this method.
8. Carboxyhemoglobin (a stable complex of carbon monoxide and hemoglobin that forms in red blood cells when carbon monoxide is inhaled, and hinders delivery of oxygen to the body, formed in carbon monoxide poisoning or tobacco smoking) and turbidity due to leukocytosis or hyperlipemia DO NOT interfere.

Expected Values - Normal range/Reference Values

Adult Males: 13.0 – 18.0 g/dL
Adult Females: 11.0 – 16.0 g/dL
Children (2 yrs to teenage): 11.0 – 16.0 g/dL
Infants (post-natal): 10 – 14 g/dL

The highest hemoglobin concentrations are usually measured in neonates. Due to the wide range of conditions (dietary, geographical, smoking, exercise, recumbency, etc.), which affect reference values, it is recommended that each laboratory establish its own expected ranges.

PROCEDURES

Performing the Blank Reading

Perform a blank reading whenever you have removed the cuvette holder (i.e., cleaning, replacing with a new cuvette holder.) You may also perform a blank reading at the start of every shift.

1. Open the cuvette holder completely.
2. The text in the display reads *"Add Cuvette"*.
3. Close the cuvette holder, without inserting a cuvette.
4. In this case the photometer does not start testing. After approx. 2 – 3 seconds the display will read "*Open Holder*". If the instrument DOES NOT show "Open Holder", there is possibly a malfunction. Please consult "Troubleshooting".
5. On your lab form, record that you performed the Blank reading.

Testing the Control Cuvette

1. Open the cuvette holder completely and wait until the photometer displays "*Add Cuvette*".
2. Take the control cuvette out of the storage box and place it in the cuvette holder.
3. Close the cuvette holder completely. *The photometer tests the control cuvette and shows the result after a few seconds.*
4. Compare the result with that stated on the storage box label. **The result must lie within the stated range**. Record the control result!
5. *If the Hgb result falls outside the range of the control cuvette, there is evidently a problem. Please consult Section 7 "Troubleshooting" in this regard.*
6. Record on the "Recording Results Form".

If the original control cuvette is lost or damaged, the photometer must be checked for proper calibration and a new control cuvette must be assayed. The photometer will need to be sent back to Stanbio Laboratory for a new control cuvette assignment. The calibration of the photometer and the re-assay of a new control cuvette is a critical and extensive process. The cost for this is the responsibility of the owner, so please take care of the control cuvette.

Performing External Quality Control

Commercial liquid controls that are recommended by Stanbio must be used on each day of testing to assure proper functioning of the entire system. Follow the manufacturer's procedure for storage and handling of the control material.

1. Allow the vials to come to room temperature 59-86°F for 20 minutes.
2. Mix thoroughly but gently by inverting the vials and repeatedly rolling them between the palms until all cellular components are completely suspended. Do not shake the vial. Check the bottom of the vial to ensure that all cells are completely suspended and not settled in the bottom of the vial. A mechanical mixer is not recommended for the procedure but can be used to maintain the cell suspension.
3. The analyzer should be in the "ready" mode prior to filling the cuvette.
4. Remove the cap from the vial. Dispense a drop of control onto a clean NON-ABSORBENT surface such as plastic film.
5. Follow Steps 14 - 18 of the testing procedure below.

6. Values should be read within **2 minutes** after filling the sample into the microcuvette.
7. While waiting for the results, clean the threads of the vial and threads of the cap with a cotton or polyester gauze. Snugly recap the vial immediately. After testing, return the vial to its proper storage.
8. Record the results on a quality control section of the "Recording Results Form".
9. Expected ranges for each level are on the package insert which comes with a specific lot number of controls. If the results do not fall within the established range, repeat the control test. If results still are outside of established ranges, consult the User's Manual or contact HemoPoint technical support at 800-531-5535. **TESTING CANNOT BE PERFORMED IF THE RESULTS OF THE CONTROLS ARE OUT OF RANGE.**

Procedure – Performing the Test

1. Make sure the Photometer is ready for use.
2. Perform the blank reading, test control cuvette (if required), and perform and record quality control.
3. Identify yourself and your patient.
4. Disinfect your hands and put on gloves.
5. Explain the procedure to your patient.
6. Make sure that your patient is sitting comfortably.
7. Remove ONE cuvette from the container and IMMEDIATELY close the lid. **NOTE:** If this is the first cuvette removed WRITE THE DATE and YOUR INITIALS on the container in the space provided. Take care NOT to touch the optical eye of the cuvette with your fingers.
8. There should be good blood circulation in the hand from which you wish to take blood, e.g., it should be warm and relaxed.
9. Lightly massage the fingers to stimulate circulation.
10. Disinfect the puncture site and allow to dry.
11. Press lightly on the fingertip with a sampling device and puncture the appropriate area of the finger.
12. WIPE AWAY the first drop of blood then, if necessary, press gently once again to get second drop of blood which is large enough to fill the microcuvette completely. AVOID "milking" the finger or using excess pressure.
13. Hold the tip of the microcuvette tip in the middle of the drop of blood and let the cavity fill from one drop by capillary action.
14. Visually inspect the cuvette for air bubbles in the optical eye. In case of air bubbles in the optical eye of the microcuvette discard and take another sample using a new microcuvette.
15. In order to avoid contamination of the cuvette holder, remove surplus blood from the outside of the microcuvette by gently wiping with a Biowipe. Do not blot the end of the cuvette.
    NOTE: The microcuvette sample prepared this way can now be measured immediately or within 10 minutes at the latest.
16. Immediately place the cuvette into the cuvette holder and close the holder. The result will be displayed within 10 seconds to 3 minutes and will remain on the display as long as the cuvette holder is in the measuring position.
17. Record the results of the hemoglobin and hematocrit USING APPROPRIATE UNITS.
18. After testing is complete, gently pull the cuvette holder out to the loading position and properly dispose of the cuvette in biohazardous trash.

Maintenance -Cleaning and disinfection of the instrument after use.

Cleaning the Housing and Touch screen

1. Disconnect the power adaptor from the electrical connection before proceeding.
2. Cleaning the housing and touch screen is best accomplished with a lint-free cloth, **lightly** dampened with clean water. For more stubborn soiling, a **mild** soap solution may be used. For disinfection, standard solutions can be used for surface disinfection provided they **do not** contain alcohol or other solvent.

Cleaning the Cuvette Holder

1. The cuvette holder can be removed from the instrument for cleaning. Proceed as follows:
    a. Disconnect the power adaptor from the electrical connection before proceeding.
    b. Open the cuvette holder until you feel a resistance and the holder will not extend further.
    c. Press down the silver pin on the left-hand side (bottom) of the cuvette holder with a ball-point pen and draw the cuvette holder forward at the same time.
2. The cuvette holder can now be cleaned with a mild soap solution. For disinfection, standard solvent-free preparations can be used. DO NOT use any cleaning agent for cleaning the cuvette holder that could leave scratches on its surface.
3. Wait until the cuvette holder is completely dry before returning it to the machine. To replace the cuvette holder, gently push it in the correct position into the opening in the housing until it engages.

Cleaning the Optical Unit

1. The optical unit is situated inside the photometer and has no direct contact with the cuvette therefore no routine cleaning is needed.
2. Cleaning the optical unit can become necessary if the measured maximum light intensity of the photometric light source no longer achieves the appropriate level required for testing.
3. For cleaning the optical unit the use of a special **Hemo**Point® **H2** optics cleaner is recommended.
4. Pull the cuvette holder out while the photometer is in the ready mode.
5. Remove the cleaner from the foil pouch and insert it (narrow tip first) carefully into the opening of the cuvette holder until you feel a smooth resistance. **THE CLEANER MUST BE USED WITHIN 10 MINUTES OF OPENING.**
6. Slowly push the cleaner deeper into the opening until it stops.
7. Wipe the optical system several times by slowly moving the cleaner in-and-out at least 5 times.
8. Remove the cleaner from the photometer.
9. If the used cleaner is dirty, repeat the procedure with a new cleaner.
10. *Wait at least 15 minutes after the optical system has been cleaned to reinsert the cuvette holder into the photometer.*
11. Dispose of all used cleaners as potentially infectious waste.

EXERCISE 7: AUTOMATED HEMOGLOBIN TESTING

Phlebotomist_____ Date_____ Points _____/**30**

Quality Control

1. Perform "blank reading" in accordance with procedure (circle one)	Yes	No

2. Test "control cuvette"	Expected Range for your cuvette:	Your reading:

Testing of Controls	Expiration Date	Expected Range (g/dL)	Value Obtained (g/dL)
3. Low: Lot #			
4. High: Lot #			
5. Based on the results of the control solutions, can this machine be used? (Circle One) Yes No			

Sample Collection and Testing (Check P for Preformed, NP for Not Preformed)	**P**	**NP**
6. Role playing: Introduce yourself		
7. Role playing: Identify the patient. Record patient name & ID number in chart below		
8. Role playing: Explain the procedure.		
9. Disinfects hands and put on gloves.		
10. Selects, prepares and organizes equipment.		
11. Selects puncture site, massages and warms site as necessary,		
12. Cleanses site, allows site to dry completely		
13. Performs an effective capillary puncture.		
14. Appropriately squeezes finger to form first drop of blood.		
15. Wipes away first drop of blood.		
16. Appropriately squeezes finger to form a second drop of blood.		
17. Fills microcuvette using second blood drop without bubbles.		
18. Applies gauze or Biowipe to puncture site, asks patient to apply pressure		
19. If necessary, wipes off excess blood from microcuvette		
20. Places microcuvette in loading tray and inserts into the machine.		
21. Reads and records results in table below		
22. Provides appropriate post-puncture care to the patient.		
23. Disposes of all materials and supplies in the appropriate containers.		
24. Inspects puncture site, applies bandage if necessary. Thanks patient.		
25. Cleans area with appropriate disinfectant.		
26. Removes gloves, washes hands.		

Recording and Evaluation of Results

Patient Name	**Patient Identification Number**	**Results in gm/dL**
27.		
28. Compared the patient results to the reference ranges stated in the lab material. Are the patient results **(CIRCLE ONE) Low Normal High**		

For Instructor Use Only:
All lines worth 1 point except for Line 27.
Line 27 is worth 3 points total; count off .5 if correct units are not used in the results space.
Count off .25 for each space where correct units are not used on lines 2, 3 and 4.

EXERCISE 7: AUTOMATED HEMOGLOBIN TESTING STUDY QUESTIONS

Name_____ Date _____ Points_____/**26**

1. Define the following terms (2.5 points):
   a. hemoglobin,

   b. anemia,

   c. polycythemia,

   d. sulfhemoglobin,

   e. carboxyhemoglobin

2. State two possible causes of low and high hemoglobin values. (2 points)
   a. Low
      i.
      ii.

   b. High
      i.
      ii.

3. State the principle of the HemoPoint H2 hemoglobin procedure. (1 point)

4. State three criteria which must be considered when setting up the HemoPoint photometer. (3 points)
   a.

   b.

   c.

5. List 3 sample types which may be used for testing. (1.5 points)

   a.

   b.

   c.

6. State the storage temperature requirements and time limits for use of blood samples. (2 points)
   a. Storage –

   b. Time limit -

7. State when a "**blank reading**" of a microcuvette is required. (1 point)

8. State the frequency of testing the "**control cuvette**". (1 point)

9. Describe the proper handling and storage of cuvettes including: temperature, amount of time they can be used after opening and the information to be placed on the container the first time opened. (3 points)
   a. Temperature
   b. Time of use after opening
   c. Information on container

10. List 4 limitations of the hemoglobin test procedure. (4 points)
    a.

    b.

    c.

    d.

11. What must be done if hemoglobin values are equal or greater than 23.5 g/dL.(1 point)

12. State the normal values for hemoglobin levels for adult females, adult males, children and infants. (2 points)
    a. Adult female
    b. Adult male
    c. Children
    d. Infants

13. State the following **time limits** (2 points total**)**:

    a. Testing the sample once it is collected in the microcuvette? (0.5 point)

    b. Vials of control to come to room temperature? (0.5 point)

    c. Optical cleaner be used after removal from the foil pouch? (0.5 point)

    d. Instrument to be used after cleaning the optical unit? (0.5 point)